THE DETERMINATION OF PARATHION AND POSSIBLE METABOLITES IN THE MILK, BLOOD AND URINE OF DAIRY COWS AFTER EXPERIMENTAL FEEDING OF PARATHION

by

JOSEPH EDWARD PANKASKIE

B. S., Kansas State College
of Agriculture and Applied Science, 1949

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

TABLE OF CONTENTS

INTRODUCTION AND LITERATURE REVIEW

Parathion is one of several chemicals originating in Germany and subsequently developed and produced commercially in this country as insecticides. The chemical, O, O-diethyl O, p-nitrophenyl thiophosphate, or parathion is an ester of thiophosphoric acid and has the empirical formula $C_{10}H_{14}NO_5PS$. Its origin in Germany has been discussed by Martin and Shaw (1946) and Thurston (1946). Early development of parathion as an insecticide is discussed by Gleissner, Wilcoxon, and Glass (1947). It is a yellow liquid when pure with a characteristic odor. Its boiling point is 375° C. at 760 mm pressure; its specific gravity is 1.26. The compound is very slightly soluble in water but is completely miscible in many organic solvents except paraffinic hydrocarbons (American Cyanamid Co. Tech. Bul. No. 2, 1949).

The increasing use during the past few years of synthetic organic chemicals for the control of insects on forage crops has led to considerable concern about the excretion of these chemicals in the milk of dairy cows. The appearance of DDT and related chlorinated hydrocarbon insecticides in the milk of cows whose diets included small amounts of these materials has been established (Arant, 1948; Biddulp et al., 1950; Carter, 1947; Carter et al., 1949; Ely et al., 1949; Lardy, 1948; Schechter et al., 1947; Shepherd et al., 1949; Smith et al., 1948; Wilson et al., 1946; and Wingo and Crister, 1949).

When parathion is used as an effective control measure for many insects attacking forage crops, a residue of less than one

part per million is left on the foliage one week or more after the last application (American Cyanamid Co. Tech. Bul. No. 2, 1949; Ginsburg et al., 1949; Ginsburg et al., 1950; Hoskins, 1949 and Hoskins, 1949a). As a basis for this study, it was considered important to determine if parathion fed in amounts equivalent to that residue would appear in the milk of dairy cows or cause deleterious effects on the health of the animals.

It has been shown (Hazleton and Holland, 1950) that when sub-lethal doses of parathion are ingested by experimental animals, it is not stored in the vital organs or the body fat of the animal. And only when nearly lethal doses are administered is the chemical excreted in the urine and then only in very small amounts. Thus, it was also considered very important to determine, if possible, the metabolic fate of the material when ingested in sub-lethal amounts.

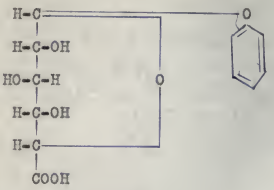
There are several interesting aspects involved in a study of the possible metabolism of parathion. Smith et al. (1932) state that certain phosphoric acid esters such as tri-o-cresyl phosphate apparently are not hydrolyzed in the alimentary canal of the cat and are absorbed only with difficulty from the alimentary canal. They arrive at this conclusion because the phenol content of urine from treated animals remains normal. Parathion is rather easily converted to p-nitrophenol and presumably diethyl thiophosphoric acid by alkaline hydrolysis and Aldridge[1], after working on the mode of action of parathion and analogs at

---

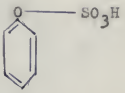
[1]Aldridge, W. M., Personal Communication, November, 1950.

the Medical Research Council, Toxicology Unit, Serum Research Institute, Carshalton, England, has suggested that parathion is hydrolyzed enzymatically in vivo to p-nitrophenol and diethyl thiophosphoric acid. A search of the literature regarding the metabolism of p-nitrophenol reveals that phenols in general are excreted through the urine in the form of conjugates with glucuronic or sulfuric acid, but especially with glucuronic acid. (Carton and Williams, 1949; Hawk et al., 1927; Malpress, 1948; Porteous and Williams, 1949; Porteous and Williams, 1949a; Torda, 1945; and Venning, 1938). It was found also that compounds such as p-phenetidine (p-ethoxyaniline) are excreted as conjugated phenols, the conjugation probably occurring after de-alkylation to the corresponding aminophenol (Smith and Williams, 1949; and Smith and Williams, 1949b). Aromatic nitro compounds may undergo reduction and subsequent acetylation and are excreted in that form (Bray et al., 1949; and Guerbet and Mayer, 1932). Thus p-acetamidobenzoic acid was isolated from urine of rabbits dosed with p-nitrobenzoic acid. Nitrophenols are excreted as conjugates of glucuronic acid and the amino derivative of the nitrophenol (Guerbet and Mayer, 1932). The basic structural formulas of these conjugates are shown below. Various substitutions may occur on the phenyl ring (Venning, 1938). Thus it can be seen that the following compounds besides parathion must be considered in a study of the metabolic fate of parathion; the amino derivative of parathion (O, O-diethyl O, p-aminophenyl thiophosphate), p-nitrophenol (both free and conjugated), p-aminophenol (both free and conjugated), N-acetyl p-aminophenol and possibly

glucuronic acid and diethyl thiophosphoric acid.



phenol glucuronide



phenyl sulfuric acid

(Formulas from Hawk et al., 1927)

EXPERIMENTAL DESIGN

Experiment I - First Capsulate Feeding of Parathion

Ten cows, including two Holsteins, four Guernseys, two Jerseys and two Ayrshires in mid-lactation were allotted to two equal groups on the basis of breed, stage of lactation and milk production. All cows were on good sudan, brome or rye pasture during the experiment. Atlas sorgo silage was fed at a level of 2 pounds per 100 pounds of body weight, and the cows had access to alfalfa hay during the time of day when they were not on pasture. A grain concentrate mixture containing approximately 16 per cent crude protein was fed according to milk production. Cows in group I were fed commercially available parathion in the form of a 25 per cent wettable powder at the level of five parts per million of the estimated dry matter intake of the roughage in the ration. Cows in group II were fed parathion at the level

of one part per million of the estimated roughage dry matter intake. The parathion was administered daily in capsules. Since forage crops treated with amounts of parathion necessary for good insect control usually contain residues of less than one part per million, the feeding levels used in this study represent an intake of parathion greater than that which normally would be ingested with parathion-treated roughage. The maximum roughage dry matter intake of the cows was estimated to be 2.25 pound per 100 pound of body weight. On this basis, cows in group I were fed 0.112 milligram of parathion per kilogram of body weight, and cows in group II were fed 0.022 milligram of parathion per kilogram of body weight. All cows were weighed on three consecutive days at bi-weekly intervals. The average of these weights was used as the basis for calculating the parathion dosage for the ensuing two week period.

These ten cows were fed parathion continuously for 81 days. In order to study the effects of parathion feeding on cows producing small amounts of milk, an additional two Ayrshires in the terminal two weeks of lactation were added, one to each of the two above groups. These two cows in late lactation were fed parathion for only 14 days. At the termination of the 81-day period, the feeding level of two cows from group I, a Guernsey and an Ayrshire, was increased immediately to 10 parts per million of parathion on the same basis as before. Then, the amount of parathion fed was doubled each succeeding week until the cows were ingesting 40 parts per million daily. At this final level, which was fed for one week, the cows were receiving approximately

0.9 milligram of parathion per kilogram of body weight daily.

Samples of carefully mixed milk were taken on alternate days for six days before the beginning of the parathion-feeding experiment and on alternate days for six days following the first feeding of parathion; after this, samples were taken twice weekly for three weeks. Thereafter, samples were taken once a week for the duration of the experiment. The milk from the Guernsey and Ayrshire cows from group I, which were fed the higher levels of parathion, was sampled and analyzed twice weekly during the three-week experiment. Milk was analyzed only for the determination of the presence or absence of parathion in this experiment.

Experiment II - Treatment and Feeding of Parathion-Treated Alfalfa

Application of Parathion to Standing Alfalfa. A 7.7 acre field plot of alfalfa located on the Agronomy Farm was obtained from the Department of Agronomy, Kansas State College. This alfalfa was sprayed with Thiophos (American Cyanamid Company), 25 per cent emulsifiable concentrate X-2, containing two pounds actual parathion per gallon of concentrate. Three gallons of the concentrate were applied to the 7.7 acre plot or an equivalent of 0.78 of a pound actual parathion per acre. The application was made on the morning of June 6, 1950, using an Essick Model 920RT compressed air sprayer with a 20-foot spray boom and an operating pressure of approximately 50 pounds per square inch. The alfalfa was cut early in the afternoon and samples were collected about 4:00 P.M. The alfalfa was raked twice to facilitate drying before baling on the morning and afternoon of June 8, 1950.

The baled alfalfa was removed immediately and stored in a barn. Fortunately, no rain fell during the entire period of application, cutting, drying, baling, and hauling.

Collection of Alfalfa Samples. Four samples of alfalfa were collected in the field immediately after cutting and were designated as west, west central, east central and east. Each of the four samples was composed of numerous (about 20) sub-samples taken at random from a section of the plot representing about one-fourth of the total area. Each section was sampled by a different worker. The samples were brought into the laboratory and 200-gram samples were taken from each section sample for analysis. These are designated as field samples in Table 1. Alfalfa from an untreated plot located on the Agronomy Farm but removed from the treated plot by one-half mile, was sampled likewise for use as untreated and standard samples (to which known amounts of parathion were added before extraction).

Three other types of alfalfa samples were collected each week during the feeding experiment for parathion determinations. One sample was taken from the approximately 20 bales that were to be fed to the cows the following week. A handful of hay was taken from each bale by jabbing an iron hook into the bale and removing a small amount of hay. An attempt always was made to equalize the amounts taken from near the center of the bale with those taken from near the outside.

During the week when these bales were fed, a sample of hay from each bale was placed in a large metal can with a tight lid. A sample from this accumulated hay was analyzed at the end of

the week and constituted the second sample. The parathion residue values for the "bale" sample from any given week should be compared to the "feeding" sample of the same week (See Table 1).

The third sample taken every week was from alfalfa that the cows refused to consume the previous week. This refused alfalfa was stored in closed barrels. A relatively large bulk of the hay was refused, partly because some of it had molded slightly. Check samples of alfalfa were taken from bales of untreated alfalfa.

Determination of Moisture Content of Alfalfa. Samples of alfalfa both from the field and from the bales were dried in an oven at 105° C. until a constant weight was obtained. By use of the weights of a sample before and after drying the per cent moisture was calculated. Treated field samples averaged 65 per cent moisture and baled samples averaged about 10 per cent moisture.

Extraction of Parathion Residue from Alfalfa. Extraction of the field samples started immediately after sampling, using a rotary tumbler previously used by Stansbury (1950). The 200-gram samples were placed in five-quart glass jars to which were added 400 milliliters of technical benzene and 20 milliliters of 10 per cent hydrochloric acid (Gunther and Blinn, 1950). The jars were capped and sealed; then extracted for one hour on a rotary tumbler. The benzene extract was then drained through coarse filter paper into a quart Mason jar. An additional 100 milliliters of benzene were added to the alfalfa in the extraction jar; the jar was capped and shaken several times. This

benzene was drained into the first extract. The recovered benzene totaled approximately 350 millliliters. The volume of extract was made up to 400 milliliters with benzene. The quart jar was sealed and set aside for analysis.

Extract Decolorization and Determination of Parathion. To each jar of extract were added 30 grams of a 2:1 mixture of Attapulgus Clay and Super-Cel. The jars were sealed again and shaken vigorously and then allowed to stand until the adsorbent settled to the bottom. The shaking process was repeated twice more. This decolorizing procedure was adopted from Gunther and Blinn (1950) to replace a more tedious chromatographic process previously suggested by Averell and Norris (1948). Occasionally, not all of the color was removed from the extract but that which was left apparently disappeared during the reduction step of the Averell and Norris (1948) method for determining parathion. Aliquots of the decolorized benzene extract were used for analysis according to the Averell and Norris (1948) procedure.

Results of Residue Analysis. About 65 per cent recovery of added known amounts of parathion of alfalfa was obtained by using the above procedure. Field samples collected about five hours after spraying had a parathion content of 86.5 parts per million based on the dry weight of the samples and 30.2 parts per million based on the wet weight of the samples. Field samples averaged 65 per cent moisture. The residue found in the bales during the 60-day feeding experiment averaged about 15 parts per million throughout the 60 days with a range from about 10 to 20 parts per million. Results of the weekly analyses of alfalfa samples

during the feeding experiment are shown in Table 1.

Alfalfa Consumption. Five dairy cows from the college herd were fed this parathion-containing alfalfa hay continuously for a period of 60 days. Feeding began 27 days after application of parathion and 25 days after the alfalfa was baled and stored. It should be pointed out again that there was a much greater amount of residual parathion on this alfalfa than would normally be found when applying parathion for effective insect control. This was true because of the extremely high rate of application of parathion and the abnormally short period of time between the application of parathion and the processing of the alfalfa. Consequently the experimental cows ingested more actual parathion than they would have by consuming alfalfa that had been treated and harvested under more normal circumstances. During this experiment weekly samples of milk, blood and urine were collected for the determination of the presence of parathion and possible metabolites.

Experiment III - Second Capsulate Feeding of Parathion

During the winter of 1950-1951 a second capsulate feeding experiment was undertaken. The purpose of this experiment was to administer even larger dosages and thereby facilitate the detection of any possible metabolite. Only one Holstein cow was fed 25 per cent parathion wettable powder in gelatin capsules. In this experiment the dosage was calculated solely on the basis of the weight of the cow. An initial dosage of one milligram of parathion per kilogram of body weight per day was administered

Table 1. Parathion residues on baled alfalfa used in the 60-day dairy cattle feeding experiment; all values are the average of at least two separate analyses carried out according to the methods of Averell and Norris (1948) and Gunther and Blinn (1950).

Week of feeding	Weight basis[1]	Parathion residue, ppm		
		Bale sample[2]	Feeding sample[3]	Refused sample[4]
1st	wet	13.4	14.5	12.0
	dry	15.2	16.2	13.4
2nd	wet	11.9	13.9	10.5
	dry	13.3	15.5	11.6
3rd	wet	18.8	13.3	17.6
	dry	20.8	14.8	19.5
4th	wet	12.7	14.0	17.0
	dry	14.1	15.5	18.8
5th	wet	12.2	10.5	14.5
	dry	13.5	11.6	16.1
6th	wet	13.8	11.9	15.3
	dry	15.3	13.1	16.9
7th	wet	11.9	14.2	13.8
	dry	13.1	15.8	15.3
8th	wet	16.3	7.7	10.0
	dry	19.0	8.5	11.1
9th	wet	11.8	14.7	9.5
	dry	13.1	16.4	10.5
Av.	wet	13.6	12.7	13.4
	dry	15.2	14.2	14.8

[1]The bale sample taken at the first week of feeding had an average moisture content of 12.5 per cent; all other samples taken during the experiment had an average moisture content of 10 per cent.

[2]A composite sample taken at the beginning of the week from approximately 20 bales that were to be fed during the week.

[3]A composite sample consisting of portions of the baled hay collected at the time of feeding throughout the week and kept in a tightly closed metal can until the end of the week when the sample was analyzed.

[4]A composite sample consisting of that alfalfa refused by the cows during the week and stored in a closed barrel until the end of the week when the sample was analyzed.

and this was increased at approximately weekly intervals until the cow was receiving a dosage of eight milligrams per kilogram per day. Urine samples were collected as needed for analysis during the experiment.

ANALYTICAL PROCEDURES

Milk Analysis

Of the various compounds involved in this study, only parathion and p-nitrophenol were studied in milk. The methods of analysis for these compounds are as follows:

Determination of Parathion. Application of the sensitive colorimetric method of Averell and Norris (1948) for estimating small amounts of parathion was tried on 100-gram samples of milk to which known amounts of parathion were added; extraction attempts were made using the methods developed by Schechter et al. (1947) and Carter (1947). The presence of interfering substances and a very low recovery of the parathion added to the milk did not permit the use of either of these established extraction methods. Upon the suggestion of Averell and Norris of the Stamford Laboratories of the American Cyanamid Company, a new procedure was tried involving the use of a liquid-liquid extraction apparatus (See Plates I and II). Essentially, this procedure involves a prolonged percolation of petroleum ether through a column of milk and ethyl alcohol. The mixture in the extraction chamber was stirred with a wire-loop stirrer inserted through the Liebig reflux condenser. The liquid-liquid extraction

EXPLANATION OF PLATE I

Diagram of liquid-liquid extraction apparatus for extracting parathion from milk.

PLATE I

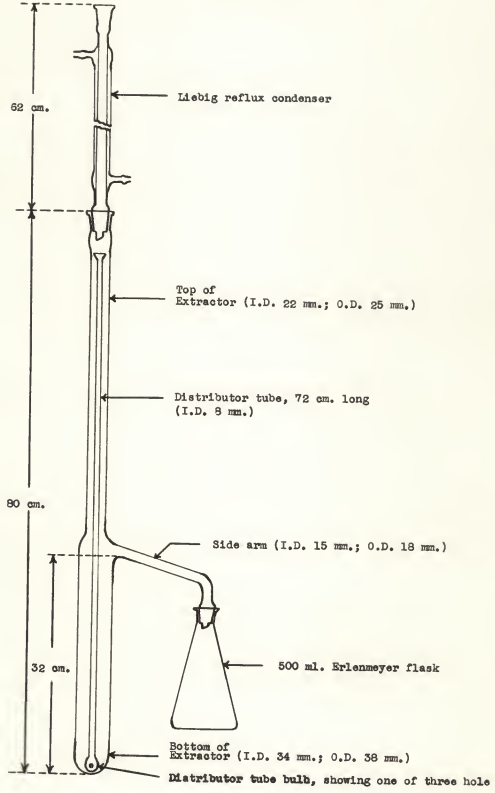


Fig. 1. EXTRACTION APPARATUS FOR THE DETERMINATION OF PARATHION IN MILK

EXPLANATION OF PLATE II

Photograph of bank of assembled liquid-liquid extraction apparatus for extracting parathion from milk.

PLATE II

procedure was carried out for an optimum period of six hours to obtain the best recovery of parathion.

Details of the analytical procedure are as follows: A mixture of 100 grams of milk and 100 milliliters of 95 per cent ethyl alcohol was placed in the liquid-liquid extraction apparatus. A pinch of NaCl, reagent grade (approximately 0.5 g) was added to the milk mixture to prevent emulsion formation in the extraction chamber and the overflowing of milk solids into the Erlenmeyer flask; 150 milliliters of technical petroleum ether (Skellysolve "B", b.p. 60-71° C.) were placed in the 500 milliliter Erlenmeyer flask of the extraction apparatus. After the extraction apparatus was assembled and mounted, the petroleum ether in the Erlenmeyer flask was heated to gentle boiling with a 175-watt electric hot plate. The solution in the extraction chamber was stirred at 30-minute intervals with the wire-loop stirrer.

After the extraction was completed, the top layer of petroleum ether in the extraction apparatus and the larger volume in the Erlenmeyer flask were transferred to a 150 milliliter beaker and evaporated almost to dryness on a warm water bath; a gentle stream of air passing over the liquid facilitated this process.

After evaporation, 20 milliliters of 95 per cent ethyl alcohol, 20 milliliters of water, 2 milliliters of 5 N HCl and 0.2 gram of zinc dust were added. The beaker was covered with a watch glass and heated to gentle boiling for 10 minutes. The beaker was allowed to cool, then the watch glass was washed with distilled water; 25 milliliters of petroleum ether were added to

the beaker and the solutions swirled around gently to extract the fat. The ether layer was siphoned off and the extraction was repeated twice more, using 25 milliliters of petroleum ether each time. After the third extraction the last traces of petroleum ether were evaporated by means of a gentle stream of air. The solution then was filtered through No. 42 Whatman filter paper into a 50-milliliter volumetric flask.

From here on the procedure is identical to that reported by Averell and Norris (1948). One milliliter of 0.25 per cent sodium nitrite was added to each flask, and the solutions were mixed well and allowed to stand for ten minutes. Then one milliliter of 2.5 per cent ammonium sulfamate was added to each flask, and the solutions were again mixed and allowed to stand another ten minutes. Finally, two milliliters of one per cent N-(1-naphthyl)-ethylenediamine dihydrochloride solution were added to each flask and water was added to make the final volume 50 milliliters. Ten minutes were again allowed for maximum color development before the per cent transmittance of each solution is taken. A milk blank was used for the 100 per cent transmittance setting. This reaction follows Beer's Law and produces a beautiful magenta color.

Standard curves were prepared from the data obtained by adding known amounts of parathion, ranging from 20 to 200 micrograms, to 100-gram samples of milk. The per cent transmittance values were obtained using a wave length setting of 555 millimicrons with a Coleman model 14 spectrophotometer. Standard curves for parathion and milk plus parathion, obtained using this procedure,

are shown in Fig. 1.

Technical petroleum ether (Skellysolve "B", b.p. 60-71° C.) was used in all the analyses. It was found that the use of petroleum ether purified according to the method reported by Werner (1933) did not improve the results. Use of 95 per cent ethyl alcohol and distilled water to dissolve the residue remaining after evaporation of the petroleum ether prevented the formation of a turbidity that developed when benzene was used. Any slight turbidity appearing upon the addition of water to the solution of the residue dissolved in ethyl alcohol either disappeared during the reduction process or was removed by filtration.

Milk blanks varied from 95 to above 100 per cent transmittance when compared with the reagent blanks; therefore, in the actual analyses no reading above 95 per cent was considered significant. It can be seen from the standardization curves for parathion plus milk that a transmittance reading of 90 per cent using a 100-gram sample of milk would represent less than 0.2 of a part per million of parathion in the milk.

Determination of p-nitrophenol (PNP). When small amounts of PNP were added to the milk and extracted in the liquid-liquid extraction apparatus, it was found that much of the PNP was extracted also with the parathion even though the solubility of PNP in petroleum ether is very slight. Therefore, efforts were concentrated on developing this method. A separate extraction procedure for PNP would involve necessarily a second sample of milk and therefore the analytical time would be increased appreciably. Thus the liquid-liquid extraction procedure was used to

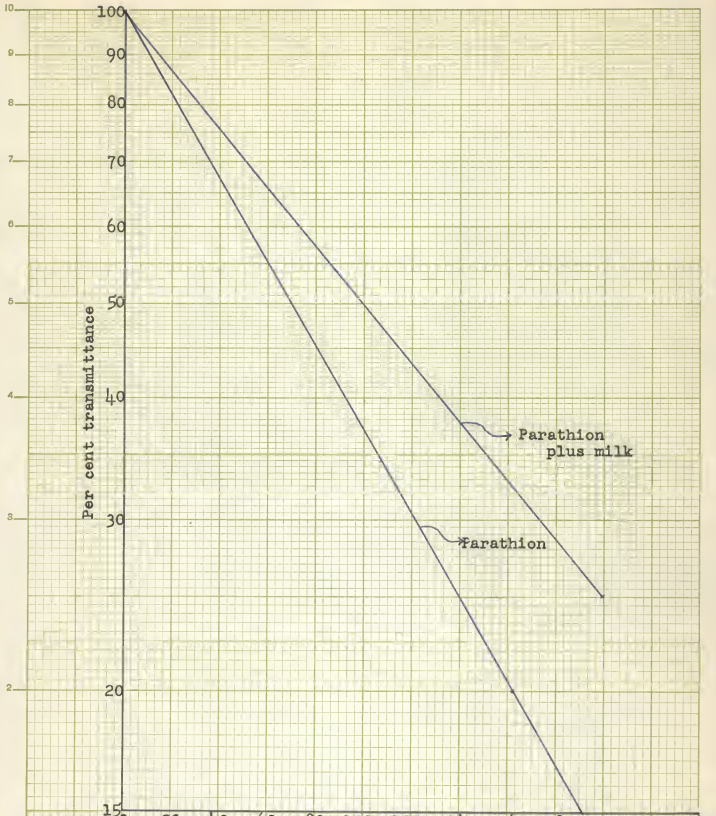


Fig. 1. Standardization curve for the determination of parathion by the Averell and Norris method using a wave length setting of 555 millimicrons on a Coleman Model 14 spectrophotometer.

remove both parathion and PNP from milk.

Averell and Geisecke of the Stamford Laboratories of the American Cyanamid Company suggested the analytical method for PNP that was used in this experiment. This method is based on the yellow color of PNP in alkaline solution and was carried out as follows: The petroleum ether extract from the liquid-liquid milk extraction process was shaken with three ten-milliliter portions of one per cent aqueous Na_2CO_3 solution in a separatory funnel. The petroleum ether fraction was saved for parathion determination. The combined aqueous fractions containing the PNP was transferred to a 50-milliliter volumetric flask. Five milliliters of 0.1 N aqueous KOH solution were added to the flask and the volume made up to the mark with water. The normality of the final solution was approximately 0.01 N. If after adding the KOH the solution was slightly turbid or cloudy, it was cleared up by adding a few milliliters of 95 per cent ethyl alcohol to the flask. The alcohol dissolved the fine particles in suspension that caused the cloudiness. The per cent transmittance of the final solution was observed on a Coleman model 14 spectrophotometer using a setting of 400 millimicrons. A milk blank was used for the 100 per cent transmittance setting. A standard curve (Fig. 2) was prepared from the data obtained by adding known amounts of PNP to the milk before extraction and analyzing the extract by the above procedure. When small amounts of PNP, ranging from 25 to 150 micrograms, were added to the milk about 60-70 per cent recovery was obtained, in comparison with the standard reagent curve.

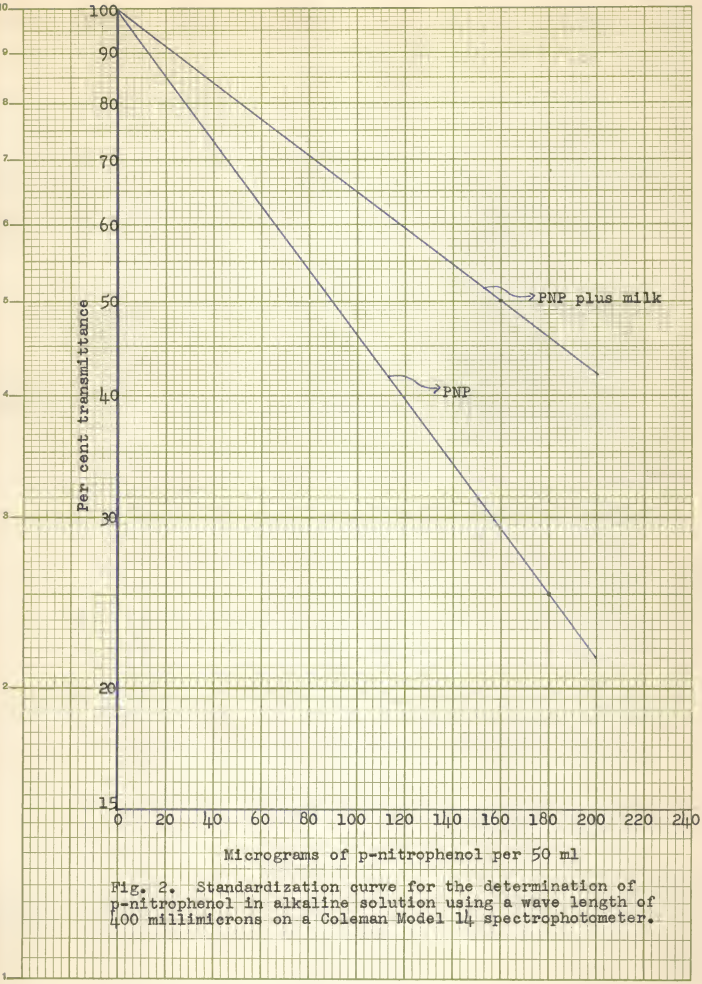


Fig. 2. Standardization curve for the determination of p-nitrophenol in alkaline solution using a wave length of 400 millimicrons on a Coleman Model 14 spectrophotometer.

The use of a 2.5 per cent sodium carbonate solution or distilled water for the separation of PNP and parathion produces an incomplete separation of the two compounds and reduces the final recovery of added amounts of parathion. The presence of both PNP and parathion in the original milk does not interfere with the determination of either of the compounds. The color intensity of the PNP in alkaline solution was not affected by varying the normality within the range of 0.006 N to 2 N KOH and possibly a wider range. This color also remains stable when left standing in alkaline solution for as long as 24 hours.

Blood Analysis

Only parathion and p-nitrophenol were considered in the analysis of blood. Both PNP and parathion were extracted from 25-milliliter samples of whole oxalated blood in a separatory funnel with three 50-milliliter portions of a 1:1 benzene:ether mixture. This solvent extract was then extracted with aqueous one per cent Na_2CO_3 solution to separate the PNP from the parathion. PNP was then determined by the method used for PNP in milk. Parathion in the benzene:ether solution was determined according to the Averell and Norris (1948) procedure. The use of the two solvents permits a recovery of 85-90 per cent of added amounts of the two materials ranging from 25 to 150 micrograms of PNP and 20 to 160 micrograms of parathion.

Urine Analysis

Quantitative methods for the determination of parathion,

p-nitrophenol and p-aminophenol in urine were developed, while only qualitative tests for glucuronic acid and the glucuronides of p-nitrophenol and p-aminophenol were possible.

Extraction. Diethyl ether appeared to be the logical solvent for use in making separatory funnel extractions of PNP, p-aminophenol (PAP) and parathion from cow's urine because of the high solubility of these compounds in this solvent and also because of the immiscibility of ether with normal urine.

A 100-milliliter sample of urine was saturated with NaCl and placed in a separatory funnel. This mixture was extracted three times with 50-milliliter portions of diethyl ether. The ether was evaporated into a few milliliters of water; this aqueous solution was used for the determination of one of the above compounds.

Determination of p-nitrophenol (PNP). The aqueous solution from above was transferred to a 50-milliliter volumetric flask. Five milliliters of an approximately 0.1 N KOH solution were added and the flask was filled to the mark with water. Transmittance readings were taken just as in the determination of PNP in milk.

Determination of Parathion. Separatory funnel extracts of normal urine made with either benzene or diethyl ether, or extracts made with petroleum ether in the liquid-liquid extraction apparatus, contain some substance that gives an interfering magenta-like color when subjected to a diazotization and coupling as in the Averell and Norris (1948) procedure for determining parathion. To avoid this interference, the possibility of

analyzing for parathion by hydrolyzing it and subsequently analyzing for its hydrolytic product, p-nitrophenol, was investigated. The separatory funnel-diethyl ether extract was transferred to a 125-milliliter Erlenmeyer flask, the ether was evaporated and the residue was redissolved in 25 milliliters of 1:1 ethyl alcohol: water mixture. Three milliliters of approximately 10 N KOH were added and the mixture was refluxed in an all-glass system for 30 minutes. The solution was cooled, transferred to a 50-milliliter volumetric flask and diluted to the mark. Any turbidity at this point was eliminated by diluting the refluxed solution with 95 per cent ethyl alcohol instead of water. Transmittance readings were taken in the same manner as for the PNP determination described above. A standard curve for parathion in terms of its hydrolytic product, p-nitrophenol, is shown in Fig. 3. Conversion from PNP to parathion was made by the following calculation:

291 = Molecular weight of parathion

139 = Molecular weight of PNP

139 micrograms of PNP $\cong$ 291 micrograms of parathion

1 microgram of PNP $\cong$ 2.093 micrograms of parathion

Therefore, the amount of parathion was found by multiplying the number of micrograms of PNP found by the factor 2.093.

Determination of p-aminophenol (PAP). Two methods for the determination of small amounts of PAP in biological tissue are reported by Brodie and Axelrod (1948). One method consists of diazotizing PAP and then coupling the diazo compound with alpha naphthol. This produces an orange color and a sensitivity of 10 micrograms is claimed. The other method involves the coupling

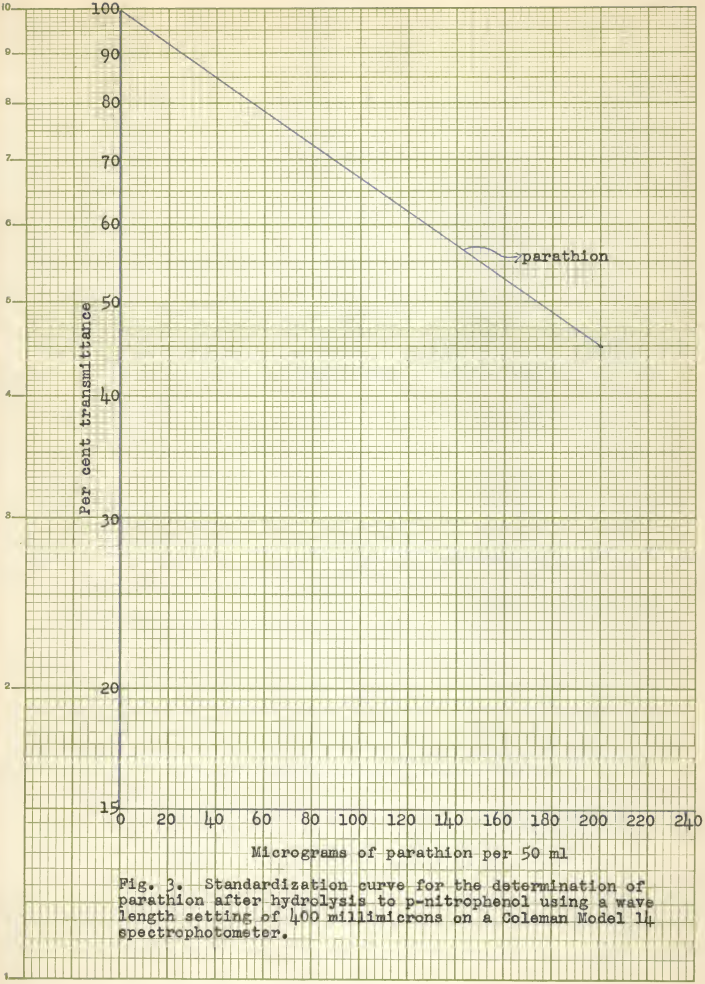


Fig. 3. Standardization curve for the determination of parathion after hydrolysis to p-nitrophenol using a wave length setting of 400 millimicrons on a Coleman Model 14 spectrophotometer.

of PAP with phenol in the presence of sodium hypobromite. This indophenol reaction produces a bright blue color and a sensitivity of two micrograms is possible. There was considerable interfering color derived from ether extracts of urine when the first of the two above methods was used. Therefore, the second method appeared more reliable. Details of this procedure are as follows: The ether extract of urine was evaporated and the residue was dissolved in five milliliters of approximately 0.01 N HCl. To this solution were added one milliliter of one per cent phenol solution and one milliliter of sodium hypobromite solution. The sodium hypobromite solution was made by adding bromine water to 1 N Na_2CO_3 solution until the mixture remained slightly yellow. The blue color in the indophenol reaction reaches its maximum density in 20 minutes. The per cent transmittance was determined on a spectrophotometer using a wave length setting of 620 millimicrons. A standard curve for p-aminophenol is shown in Fig. 4.

Determination of Conjugated Phenols or Glucuronides. All the analytical methods for conjugated phenols in the literature are based on the hydrolysis of the conjugated phenols into the phenol and glucuronic acid and the subsequent determination of that phenol and of glucuronic acid. Brodie and Axelrod (1948) report a method of hydrolyzing conjugated phenols consisting essentially of heating a small amount of biological fluid in concentrated HCl under pressure. A more convenient procedure is reported by Porteous and Williams (1949). In this procedure concentrated H_2SO_4 is added to urine until the resulting mixture is equivalent to about 10 N acid. This mixture is then refluxed

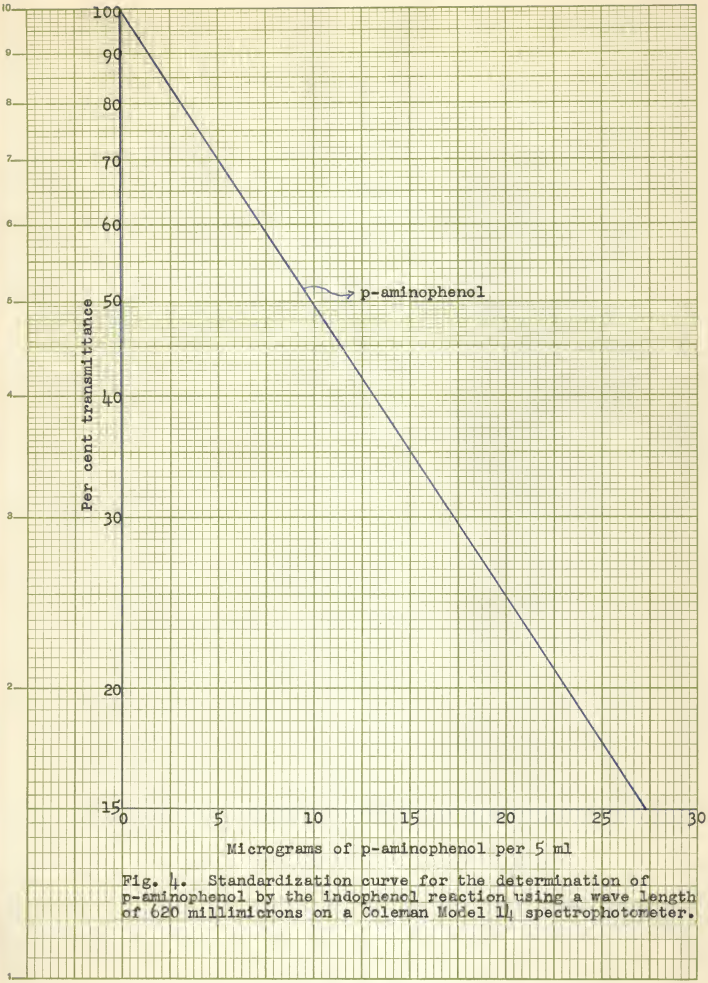


Fig. 4. Standardization curve for the determination of p-aminophenol by the indophenol reaction using a wave length of 620 millimicrons on a Coleman Model 14 spectrophotometer.

for one hour. This method of hydrolysis was used both on whole urine and on ether extracts of urine. After hydrolysis was complete the hydrolysate was neutralized with NaOH and buffered with K_2HPO_4 and then extracted with diethyl ether to remove the phenol. This ether extract was re-extracted with five milliliters of approximately 0.01 N HCl and the acid extract was used for PAP determination.

Determination of Glucuronic Acid. The method for detecting glucuronic acid was the naphthoresorcinol reaction reported by Hawk (1927) and Hanson et al. (1944). The reaction produces a blue color. Details of the procedure are as follows: Ten milliliters of urine, one milliliter of a one per cent alcoholic solution of naphthoresorcinol and 10 milliliters of concentrated HCl were mixed in a test tube and heated to boiling for 15 minutes. The solution was cooled and then extracted with 25 milliliters of diethyl ether. The per cent transmittance of the ether solution was taken using a wave length setting of 570 millimicrons. The presence of glucuronides was indicated by a lavender-blue color.

Determination of Diazotizable Material. This procedure will be referred to as the diazo reaction. It is an adaptation of the diazotization and coupling reactions used in the Bratton and Marshall (1939) procedure and the Averell and Norris (1948) procedure. This procedure was used both on 5-milliliter samples of whole urine and on ether extracts of 100-milliliter samples of urine. In the latter case the ether was evaporated and the residue was taken up in water which was transferred to a 50-milliliter

volumetric flask. If whole urine was used it was placed directly in the volumetric flask. To the solutions in the flask were added two milliliters of 5 N HCl, the solution was cooled and one milliliter of 0.25 per cent sodium nitrite was added. The solution was mixed well and allowed to stand 10 minutes. Then one milliliter of 2.5 per cent ammonium sulfamate was added to the flask and the solutions were again mixed and allowed to stand another 10 minutes. Finally, two milliliters of one per cent N-(1-naphthyl)-ethylenediamine dihydrochloride solution were added to the flask and water was added to make the final volume 50 milliliters. Ten minutes was again allowed for maximum color development before the per cent transmittance of each solution was taken. A urine blank was always used for the 100 per cent transmittance setting at a wave length setting of 555 millimicrons. A color was obtained when normal urine was subjected to this reaction but analysis of urine from six separate check cows indicated that the concentration of diazotizable material in normal urine is quite uniform among different cows so that any increase in the color density from urine from experimentally-fed cows might be suspected of being due to a metabolite of parathion. Suspected metabolites that will give a positive diazo reaction are the amino derivative of parathion and p-aminophenyl glucuronide (Smith and Williams, 1949a).

Fecal Analysis

Determination of Parathion. Fresh feces were collected from the cow receiving parathion along with feces from a check

cow. The fecal samples were analyzed for parathion according to the following procedure: A mixture of 100 grams of feces and 100 milliliters of distilled water in a quart Mason jar was made into a slurry and then 400 milliliters of benzene was added. The jar was capped tightly and rotated on a mechanical tumbler for one hour. The benzene was decanted off and aliquots were used for parathion determination according to the Averell and Norris (1948) procedure.

ANALYTICAL RESULTS

Milk Analysis

Parathion. No parathion was ever found in the milk of cows receiving parathion either in capsules or as a residue on alfalfa hay. Approximately 250 separate analyses for parathion in the milk were made during the course of the first capsulate-feeding experiment and about 70 analyses were made on milk of cows fed the parathion-treated alfalfa.

p-nitrophenol. No p-nitrophenol was found in the milk of the cows fed the parathion-treated alfalfa hay. About 70 analyses of milk from these cows covering a period of 80 days were made.

Diazotizable Material. No diazotizable material was ever found in the petroleum extracts of milk of experimentally fed cows.

Blood Analysis

Parathion, p-nitrophenol and Diazotizable Material. None

of these suspected metabolites was ever found in the benzene: ether extracts of whole blood from cows fed parathion-treated alfalfa hay.

Urine Analysis

Parathion. No parathion was found in the urine of cows fed parathion either in capsules or as a residue on alfalfa hay.

p-nitrophenol. Likewise no p-nitrophenol, either free or conjugated, was found in the urine of experimentally-fed cows.

Diazotizable Material. A positive diazo reaction is obtained both on ether extracts of 100-milliliter urine samples or on 5-milliliter samples of whole urine. Colored check samples resulted as reported in the procedure but the color in the check samples was not as dense as the color in the samples from cows fed either capsulate parathion or parathion-treated alfalfa hay. This increased color intensity persisted throughout the course of the parathion-treated hay-feeding experiment and then disappeared after the cows were put on a normal diet. Then when one cow was again fed parathion in capsules the increased color again appeared and as the dosage of this cow was increased the color intensity increased while the color intensity from the urine of the check cow remained normal. Efforts to separate the normal color-producing constituents of the urine from the constituents causing the more dense color in the urine from the experimentally-fed cow were unsuccessful. About the same color difference is obtained from 5-milliliter samples of whole urine as is obtained from ether extracts of 100-milliliter samples of urine. There-

fore, it appears that only a small amount of the color-producing substance is extractable with ether.

The diazo colors derived from parathion, from check urine and from urine from experimentally-fed cows are all apparently the same color and have identical absorption curves with absorption peaks at a wave length of 555-560 millimicrons. The compound causing the extra color in the experimentally-fed cows cannot be parathion because no reduction step is needed before diazotization can be accomplished. Also p-nitrophenol cannot be detected after alkaline hydrolysis as outlined in the procedure for determination of parathion in urine. The color could be due to p-aminophenylglucuronide or to the amino derivative of parathion.

p-aminophenol. No free p-aminophenol was detected in ether extracts of urine.

Conjugated p-aminophenol. After either the ether extracts or whole-urine samples are subjected to strong acid hydrolysis and further treated as in the procedure for glucuronide hydrolysis, a positive test for p-aminophenol can be obtained. This positive test does not necessarily indicate the positive presence of p-aminophenylglucuronide as the amino derivative of parathion would also be hydrolyzed to p-aminophenol when subjected to such a severe hydrolytic treatment. When small volumes of whole urine were hydrolyzed previous to the determination of p-aminophenol about five micrograms of p-aminophenol per milliliter of urine was found in the urine collected during the week when two milligrams per kilogram per day was administered to one cow. When seven milligrams per kilogram per day was administered about 15

micrograms p-aminophenol per millliliter of urine was found.

Glucuronic Acid. Normal urine contains a small amount of glucuronic acid and therefore a positive test, i.e., the lavender-blue color is obtained on normal urine. However, if normal urine is used for the 100 per cent transmittance reading on the spectrophotometer readings of about 30 per cent are obtained on samples of urine from the cow dosed with parathion. Therefore, the presence of excess glucuronic acid is indicated.

Isolation of p-aminophenylglucuronide. The following procedure reported by Smith and Williams (1949a) was used in attempts to isolate crystalline p-aminophenylglucuronide from cow's urine. Twenty liters of urine from the cow dosed with parathion were processed to obtain the final solution. The urine was made faintly acid with acetic acid and treated with saturated normal lead acetate. The precipitate was removed and discarded. The filtrate was made faintly alkaline and basic lead acetate added until precipitation was complete. The basic lead precipitate was filtered and washed with water. It was then suspended in water and the lead removed with H_2S. The PbS was removed by filtration and the filtrate was treated with cold saturated mercuric acetate solution. A pinkish precipitate separated and was filtered off. It was suspended in water and the mercury removed with H_2S. The suspension was filtered to remove the HgS and the filtrate was evaporated at reduced pressure to 15 milliliters and this was brought to pH 4.3 with 0.5 M sodium acetate. The mixture was kept at 0° overnight. Smith and Williams (1949b) were able to separate a crystalline precipitate of p-aminophenyl-

glucuronide from this cold solution in their experiments with urine from rabbits dosed with aniline.

No p-aminophenylglucuronide could be induced to precipitate from the final cold filtrate of cow's urine; however, p-aminophenol and glucuronic acid could be detected colorimetrically in the final solution after acid hydrolysis. No p-aminophenol could be found in the final solution when normal or check urine was processed according to the above procedure. The p-aminophenol above probably is a hydrolytic product of p-aminophenylglucuronide because the glucuronide is the only possible metabolite that should appear in the final filtrate. The amino derivative of parathion would not be precipitated by lead or mercury and consequently would not appear in the final filtrate.

Fecal Analysis

Parathion. Feces from the cow receiving six milligrams of parathion per kilogram per day contained parathion at the level about two parts per million. The reduction step is necessary before a positive colorimetric reaction is obtained; therefore, the compound in feces is probably parathion.

DISCUSSION

Parathion, as a residue on alfalfa hay and in capsules, has been fed to dairy cows in amounts much greater than the amount of parathion which dairy cows might ingest as a residue on forage crops when these forage crops had been sprayed with parathion according to current insect control recommendations.

There were no harmful effects on the health of the cows after rather extensive feeding of large doses of parathion. No parathion was ever found in the milk of cows in the first two experiments. These two facts indicate that crops sprayed with parathion at the usual rates are quite safe for dairy cattle feed and that the milk from these cows is safe for human consumption.

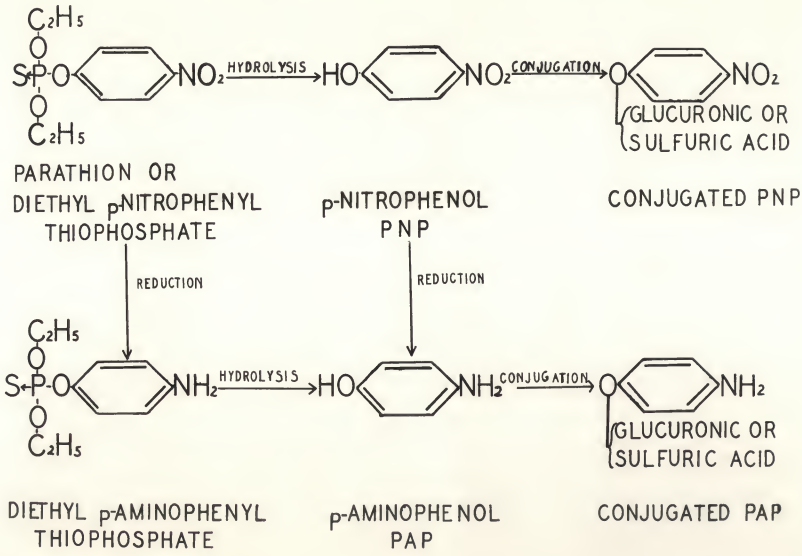
The question of the metabolic fate of parathion in dairy cows is a more difficult one. Analytical results indicate that at least a portion of the dosage is excreted in the urine as p-aminophenylglucuronide. Even though p-aminophenylglucuronide could not be isolated in crystalline form from large volumes of urine, the detection of p-aminophenol in the urine only after strong acid hydrolysis and the detection of excess glucuronic acid in the urine of the cow dosed with parathion indicate that before hydrolysis these two compounds existed as p-aminophenyl-glucuronide. The fact that p-aminophenol could be found in the final filtrate after processing the urine for the isolation of p-aminophenylglucuronide lends support to this theory. It can be said quite definitely that the metabolite is a diazotizable primary aryl amine and that it is p-aminophenol in combination with some other substance in such a manner so that strong acid hydrolysis releases free p-aminophenol. A possible route of metabolism is shown on Plate III. However, assuming an average of 15 kilograms urine excreted per day and an average cow weight of 650 kilograms, calculations show that only about 10 per cent of the dosage of parathion is accounted for by the p-aminophenol content

EXPLANATION OF PLATE III

Diagram of possible routes of metabolism of parathion in cows.

PLATE III

POSSIBLE METABOLISM OF PARATHION



of hydrolyzed urine. Assuming an average fecal deposit of 35 kilograms only two per cent of the dosage is accounted for as parathion eliminated in the feces. It is conceivable that this much of the dosage is mechanically swept through the alimentary tract before it can be absorbed and metabolized.

One other fact bears noting. Parathion is considered to be quite volatile as far as residues are concerned. In this experiment, parathion residues disappeared quite rapidly during the period between the application and storage of the bales but after the bales were stored in a barn the residue in the bales appeared to be quite stable at least for the feeding period of 60 days.

SUMMARY AND CONCLUSIONS

Three experiments in which dairy cows were fed parathion as a wettable powder in capsules and as a residue on alfalfa hay have been completed.

Alfalfa sprayed with parathion and subsequently cut, baled and stored in a barn had a parathion residue of about 15 parts per million while it was fed to dairy cows.

No harmful effects on the health of the cows were noted at any time during rather extensive feeding of parathion.

No parathion or p-nitrophenol was found in the milk of cows ingesting parathion as a residue on alfalfa hay.

No parathion, p-nitrophenol, or p-aminophenol was found in the blood or urine of dairy cows ingesting parathion.

Analytical results indicate that at least a part of the parathion ingested by dairy cows is excreted through the urine as p-

aminophenylglucuronide.

A very small amount of parathion is eliminated through the feces.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Paul A. Dahm under whose able direction the work was done, and to Dr. Roger C. Smith for guidance and encouragement throughout the course of the project and especially for his guidance in the use of literature and in manuscript preparation.

The author is also grateful to Professor F. W. Atkeson and Dr. F. C. Fountaine of the Department of Dairy Husbandry for their cooperation in planning the experiments and for providing for the care of the dairy cows and collection of all the samples.

To the American Cyanamid Company who supplied certain equipment and the funds without which the work would not have been possible and also to Drs. Averell, Norris and Giesecke of the above company for technical assistance, the author is deeply indebted.

LITERATURE CITED

American Cyanamid Company. Agricultural Chemicals Division. Technical Bulletin No. 2. 1949.

Arant, F. S.
Status of velvet bean caterpillar control in Alabama. Jour. Econ. Ent. 41:26-30. 1948.

Averell, P. R. and M. V. Norris.
Estimation of small amounts of 0,0-diethyl 0, p-nitrophenyl thiophosphate. Anal. Chem. 20:753-756. 1948.

Biddulph, C., G. Q. Bateman, M. J. Bryson, J. R. Harris, D. A. Greenwood, W. Binns, M. L. Miner, L. E. Harris, and L. L. Madsen. DDT in milk and tissues of dairy cows fed DDT-dusted alfalfa hay. Agricultural Control Chemicals, American Chemical Society, Advances in Chemistry Series, No. 1: 237-244. 1950.

Bratton, A. C. and E. K. Marshall.
A new coupling component for sulfanilamide determination. Jour. Biol. Chem. 128:537-550. 1939.

Bray, H. G., W. V. Thorpe, and P. G. Wood.
The fate of certain organic acids and amides in the rabbit. Biochem. Jour. 44(1):39-45. 1949. (Other references included)

Brodie, Bernard B. and Julius Axelrod.
The estimation of acetanilide and its metabolic products, aniline, N-acetyl-p-aminophenol, and p-aminophenol (free and total conjugated) in biological fluids and tissues. Jour. Pharmacol. and Expt. Therap. 94(1):22-28. 1948.

Carter, R. H.
Estimation of DDT in milk by determination of organic chloride. Indus. and Engg. Chem., Analyt. Ed., 19:54. 1947.

Carter, R. H., P. E. Hubanks, H. D. Mann, F. F. Smith, P. G. Piquett, J. C. Shaw, and L. P. Ditman. The DDT content of milk from cows fed pea vine silage containing DDT residues. Jour. Econ. Ent. 42:119-122. 1949.

Dahm, Paul A., F. C. Fountaine, J. E. Pankaskie, Roger C. Smith and F. W. Atkeson. The effects of feeding parathion to dairy cows. Jour. Dairy Sci. 33(10):747-757. (Publication of a portion of the thesis previous to submission of thesis approved by Graduate Council)

Ely, R. E., L. A. Moore, R. H. Carter, and P. W. Poos.
The DDT, toxaphene, and chlordane content of milk as affected by feeding alfalfa sprayed with these insecticides. U.S.D.A., Agr. Research Admin. BDIM-Inf-85. 4 p., Dec. 1949.

Garton, G. A. and R. T. Williams.
Studies in detoxication 21. The fates of quinol and resorcinol in the rabbit in relation to metabolism of benzene. Biochem. Jour. 44(2):234-238. 1949.

Ginsburg, J. M., R. S. Filmer, J. P. Reed, and A. R. Paterson.
Recovery of parathion, DDT and certain analogs of dichloro-diphenyl-dichloroethane from treated crops. Jour. Econ. Ent. 42(4):602-611. 1949.

Ginsburg, J. M., R. S. Filmer, and J. P. Reed.
Longevity of parathion, DDT, and dichloro-diphenyl-dichloro-ethane residues of field and vegetable crops. Jour. Econ. Ent. 43(1):90-94. 1950.

Gleissner, Bruce D., Frank Wilcoxon, and Edward H. Glass.
O,O-diethyl O-p-nitrophenyl thiophosphate---A promising new insecticide. Agr. Chemicals 2:61. 1947.

Guerbet, Maurice and Andre Mayer.
Pharmaco-dynamic action of nitrated phenols. Ann. Physiol. Physicochim. Biol. 8:117-121. 1932. (Abstract only)

Gunther, F. A. and R. C. Blinn.
Mass-production techniques for estimation of parathion residues. Agricultural Control Chemicals, American Chemical Society, Advances in Chemistry Series, No. 1: 72-87. 1950.

Hanson, S. W. F., G. T. Mills, and R. T. Williams.
A study of the determination of glucuronic acid by the naphthoresorcinol reaction, with photoelectric absorptiometer. Biochem. Jour. 38:274-278. 1944.

Hawk, Philip B., Bernard L. Oser, and William H. Summerson.
Practical Physiological Chemistry 12 ed. Philadelphia, The Blakiston Co. 1927.

Hazleton, Lloyd W. and Emily G. Holland.
Pharmacology and toxicology of parathion. Agricultural Control Chemicals, American Chemical Society, Advances in Chemistry Series, No. 1: 31-38. 1950.

Hoskins, W. M.
How pest control programs affect residues in foods. Agr. Chemicals 4(10):22-27, 77-78. 1949.

Hoskins, W. M.
Deposit and residue of recent insecticides resulting from various control practices in California. Jour. Econ. Ent. 42(6):966-973. 1949a.

Lardy, H. A.
Experiments with peas and sweet corn treated with DDT insecticides. Indus. and Engg. Chem., 40:710-711. 1948.

Malpress, F. H.
Colorimetric estimation of stilbestrol, hexestrol and their glucuronides in urine. Biochem. Jour. 43:132-136. 1948.

Martin, H. and H. Shaw.
Developments in methods and materials for the control of plant pests and diseases in Germany. BIOS Final Rept. No. 1095, Item No. 22, British Intelligence Objectives Subcommittee, 1946.

Porteous, J. W. and R. T. Williams.
Studies in detoxication 19. The metabolism of benzene. Biochem. Jour. 44(1):46-55. 1949. (Other references included)

Porteous, J. W. and R. T. Williams.
Studies in detoxication 20. The metabolism of benzene. Biochem. Jour. 44(1):56-61. 1949a.

Schechter, M. S., M. A. Pogorelskin, and H. L. Haller.
Colorimetric determination of DDT in milk and fatty materials. Indus. and Engg. Chem., Analyt. Ed., 19:51-53. 1947.

Shepherd, J. B., L. A. Moore, R. H. Carter, and F. W. Poos.
The effect of feeding alfalfa hay containing DDT residue on the DDT content of cow's milk. Jour. Dairy Sci. 32:549-555. 1949.

Smith, Maurice I., E. W. Engel, and E. F. Stohlman.
Further studies on the pharmacology of certain phenol esters with special reference to the relation of chemical constitution and physiologic action. Nat'l. Inst. Health Bul. No. 160. 1932.

Smith, R. F., W. M. Hoskins, and O. H. Fullmer.
Secretion of DDT in milk of dairy cows fed low-residue alfalfa hay. Jour. Econ. Ent. 41(5):759-763. 1948.

Smith, J. N. and R. T. Williams.
Studies in detoxication 22. The metabolism of phenacetin (p-ethoxy acetanilide) in the rabbit and further observations on acetanilide metabolism. Biochem. Jour. 44(2):239-242. 1949.

Smith, J. N. and R. T. Williams.
Studies in detoxication 23. The fate of aniline in the rabbit. Biochem. Jour. 44(2):242-250. 1949a.

Smith, J. N. and R. T. Williams.
Studies in detoxication 24. The metabolism of p-phenetidine (p-ethoxy aniline) with some observations on the anisidines (methoxy-anilines). Biochem. Jour. 44(2):250-255. 1949b.

Stansbury, R. E.
The effect of alfalfa dehydration upon residues of aldrin, chlordane, parathion, and toxaphene. Unpublished master's degree thesis. Kansas State College, Manhattan, 1950.

Thurston, J. T.
Organic chemical intermediates for insecticides, fungicides and rodenticides. FIAT Final Rept. 949. U. S. Dept. of Commerce. 1946.

Torda, C.
Influence of low concentrations of cocaine on the metabolism of phenol. Jour. Pharmacol. 78:340-345. 1945.

Venning, Eleanor Hill.
Further studies on the estimation of small amounts of sodium pregnanediol glucuronidate in urine. Jour. Biol. Chem. 126: 595-602. 1938.

Wilson, H. F., N. N. Allen, G. Bohstedt, J. Bethell, and H. A. Lardy. Feeding experiments with DDT-treated pea vine silage with special reference to dairy cows, sheep, and laboratory animals. Jour. Econ. Ent. 39:801-806. 1946.

Wingo, C. W. and O. S. Crister.
Effect of DDT on dairy cattle and milk. Jour. Econ. Ent. 41(1):105-106. 1948.

THE DETERMINATION OF PARATHION AND POSSIBLE METABOLITES IN THE MILK, BLOOD AND URINE OF DAIRY COWS AFTER EXPERIMENTAL FEEDING OF PARATHION

by

JOSEPH EDWARD PANKASKIE

B. S., Kansas State College
of Agriculture and Applied Science, 1949

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

Three experiments in which dairy cows were fed parathion as a wettable powder in capsules and as a residue on alfalfa hay are reported.

Alfalfa sprayed with parathion at the rate of 0.78 pound per acre and subsequently cut, baled and stored in a barn had a parathion residue of about 15 parts per million while it was fed to dairy cows.

A review of the literature reveals that parathion is probably hydrolyzed in vivo to p-nitrophenol and diethyl thiophosphoric acid. Phenols in general are excreted through the urine as conjugates with glucuronic acid and nitrophenols are reduced to the corresponding aminophenol and then excreted as conjugates with glucuronic acid.

Colorimetric analytical methods are given for parathion and p-nitrophenol in milk, for parathion and p-nitrophenol in blood, for parathion, p-nitrophenol, p-aminophenol, conjugated phenols or glucuronides and glucuronic acid in urine and for parathion in feces.

No harmful effects on the health of the cows were noted at any time during rather extensive feeding of parathion.

No parathion or p-nitrophenol was found in the milk of cows ingesting parathion as a residue on alfalfa hay.

No parathion, p-nitrophenol, or p-aminophenol was found in the blood or urine of dairy cows ingesting parathion.

Analytical results indicate that at least a part of the parathion ingested by dairy cows is excreted through the urine

as p-aminophenylglucuronide.

A very small amount of parathion is eliminated through the feces.